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Effect of pre-treatment with 5-bromouracil in an ultraviolet sensitive strain of *Neurospora crassa*

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SUMMARY

5-Bromouracil was shown to enhance the mutation frequency in an ultraviolet sensitive strain of *Neurospora crassa* (*uvs*-2) that was previously irradiated with ultraviolet light. The design of the experiment eliminated light or dark repair as possible hypotheses to account for the observed enhancement. Since a similar effect was noted at the *rib*-1 locus in *N. crassa*, which is not a UV-sensitive strain, it appears that the error prone repair mechanism in *N. crassa* may be related to post-replication repair. This enhancement may be attributed to the interference of 5-bromouracil with post-replication repair. It is also postulated that the maximum increase in the mutation frequency occurs when the greatest number of unrepaired pre-mutational UV lesions are repaired in the presence of 5-bromouracil.

1. INTRODUCTION

Ultraviolet light causes mutation by errors introduced during repair of pyrimidine dimers on the same DNA strand, the most frequent type of which is thymine-thymine pairs (Witkin, 1969*a*). Several different repair mechanisms have been described which are involved in the repair of these lesions (Worthy & Epler, 1973*a*; Worthy & Epler, 1972). The first, termed photoreactivation, involves a light activated enzyme which splits the dimer *in situ* (Witkin, 1966). A second process known as excision repair enzymically excises the offending dimer, along with nucleotides on either side (Boyce & Howard-Flanders, 1964; Setlow & Carrier, 1964). A repair synthesis then occurs where the opposing chain serves as a template for the repolymerization of the missing nucleotides, after which the DNA backbone is sealed by a ligase (Howard-Flanders & Boyce, 1966). Both photoreactivation and excision repair are known to be quite accurate, resulting in relatively few errors (Witkin, 1966). The third mechanism, termed post-replication repair, is described as being less faithful, repair not taking place until after the first replication of DNA (Witkin, 1969). The dimer is repaired by a recombination like process, involving pairing between two daughter strands each of which utilizes the other as a template to fill in gaps opposite unexcised dimers. The

mutation frequency caused by errors in this process is greater than for either light or excision repair. UV-sensitive strains of *N. crassa* differ in their ability to perform these error correcting operations (Catcheside, 1974).

Experiments conducted by Aoki, Boyce & Howard-Flanders (1966) in which bacteria were first incubated in 5-bromouracil (5-BU) and later exposed to UV, led to the conclusion that 5-BU incorporation into DNA prior to UV exposure increases sensitivity to radiation. The increase in UV sensitivity has been attributed to the inhibition of DNA synthesis during excision repair. Schroeder (1974) described a series of studies indicating that similar systems might be operating in prokaryotes and eukaryotes that affect DNA repair and mutation.

In a study of the interactive effect of UV and 5-BU at the *rib*-1 locus in *N. crassa*, Nemerofsky (1975) demonstrated that 5-BU enhanced the reversion frequency when added to conidia of a riboflavin requiring, temperature-sensitive mutant of *N. crassa* that were first irradiated with UV light. Possible explanations for the observed enhancement at the *rib*-1 locus excludes interference in light repair since photoreactivating light was eliminated. Two hypotheses may account for the observation: (1) 5-BU may inhibit excision or synthesis during excision repair, resulting in the persistence of unrepaired UV lesions; or (2) 5-BU interferes with post-replication repair thereby decreasing its accuracy.

To investigate the possible mechanism of the interactive effect between 5-BU and UV light, a UV-sensitive strain of *N. crassa*, designated as *uvs*-2 by Stadler & Smith (1968) was employed. They found *uvs*-2 to be 10 times more sensitive to UV light than wild type *Neurospora* and has no effect in recombination. De Serres (1971) and Witkin (1969*a*) reported that *uvs*-2 also showed an increase in UV mutability. The demonstration that *uvs*-2 is normal in recombination and shows an increase in UV mutability is consistent with the finding that the error prone repair system in *E. coli* is post-replication, and also supports the contention that the error prone system in *Neurospora* is post-replication repair (Schroeder, 1974).

Since *uvs*-2 lacks excision repair, and if the observed enhancement between UV and 5-BU in *rib*-1 is due to the interference of 5-BU on excision repair, no enhancement would be observed in a system employing *uvs*-2. However, if enhancement is observed in *uvs*-2, the implication is that 5-BU affects post replication repair in this strain when photo-reactivating light is excluded. *uvs*-2 conidia were first exposed to different doses of UV light and then incubated in media containing 5-BU. Survival values were obtained, and the mutation frequency was calculated from the frequency of auxotrophic survivors at a given UV dose and BU treatment.

2. MATERIALS AND METHODS

Conidia were collected using sterile distilled water from a 5–7 day old flask of *uvs*-2 (obtained from Fungal Genetics Stock Center, Arcata, Calif.) growing on Westergaard–Mitchell minimal Media (W-M) (Westergaard & Mitchell, 1947). Conidia were filtered once through four layers of cheese cloth and twice through

glass wool. Conidia were then counted in a haemacytometer and an aliquot transferred to a sterile petri dish. Background illumination was supplied by a G.E. 'Gold' lamp (F 15 T8 G0). The conidal suspension was irradiated with constant stirring on a magnetic stirrer, using a 15 W G.E. germicidal lamp (G15-T8) as a UV source at a rate of 0·8 J/m². Samples of irradiated conidia were transferred to sterile screw-top test-tubes and centrifuged. The conidia were then resuspended and incubated in 17 ml of W-M supplemented with 1 g of vitamin-free casamino acids (Difco) and 1 ml of synthetic vitamin mixture (Tatum *et al.* 1950). This initial incubation interval is referred to as pre-incubation.

Following pre-incubation the conidia were centrifuged, resuspended in 17 ml of supplemented W-M containing 0·41 mg 5-BU/ml and incubated at 25 °C for a second time. This second incubation is referred to as post-incubation. To remove the 5-BU, the suspension was washed 3 times by centrifugation and resuspended in 12 ml of distilled water. Conidia were finally plated on sorbose supplemented media (Brockman & de Serres, 1963) at 37 °C, and 72 h later the colonies that

Table 1. *N/S ratio for uvs-2 following UV irradiation and 5-BU treatment**

UV dose in J/m²	No BU treatment	30 min pre-incubation incubation time (min) in BU		15 min pre-incubation incubation time (min) in BU	
		15	30	15	30
67·2	3·67	8·58	8·47	2·87	8·80
76·8	11·61	20·70	24·66	24·38	38·72
86·4	23·45	11·08	16·34	19·38	29·14

* Average of three experiments.

appeared were picked and isolated on sucrose supplemented media. The surviving colonies were subsequently growth tested on liquid sucrose minimal at 37 °C and scored as auxotrophic mutants if they failed to grow, or showed only limited growth.

3. RESULTS

The sensitivity of *uvs-2* to UV light is presented in Fig. 1. A comparison is presented in Table 1 between various incubation combinations in the presence and absence of 5-BU while varying the UV dose. The mutation frequency is presented in Fig. 2, where the ratio N/S is plotted against UV dose. The value of N/S was obtained by dividing the number of auxotrophs among the total isolated from a given BU treatment (N) by the survival value at that UV exposure (S). The N/S ratio allows for a comparison of mutation frequency corrected for the survival value at a given treatment. It appears that the maximum net increase occurs at a UV dose of 76·8 J/m² when compared to a UV dose of 67·2 or 86·4 J/m². A UV dose of 96·0 J/m² showed no significant difference between zero BU treatment and treatment with BU. The detection of mutants is non-selective, in that all viable conidia that are expressed on the sorbose plates are collected and growth tested.

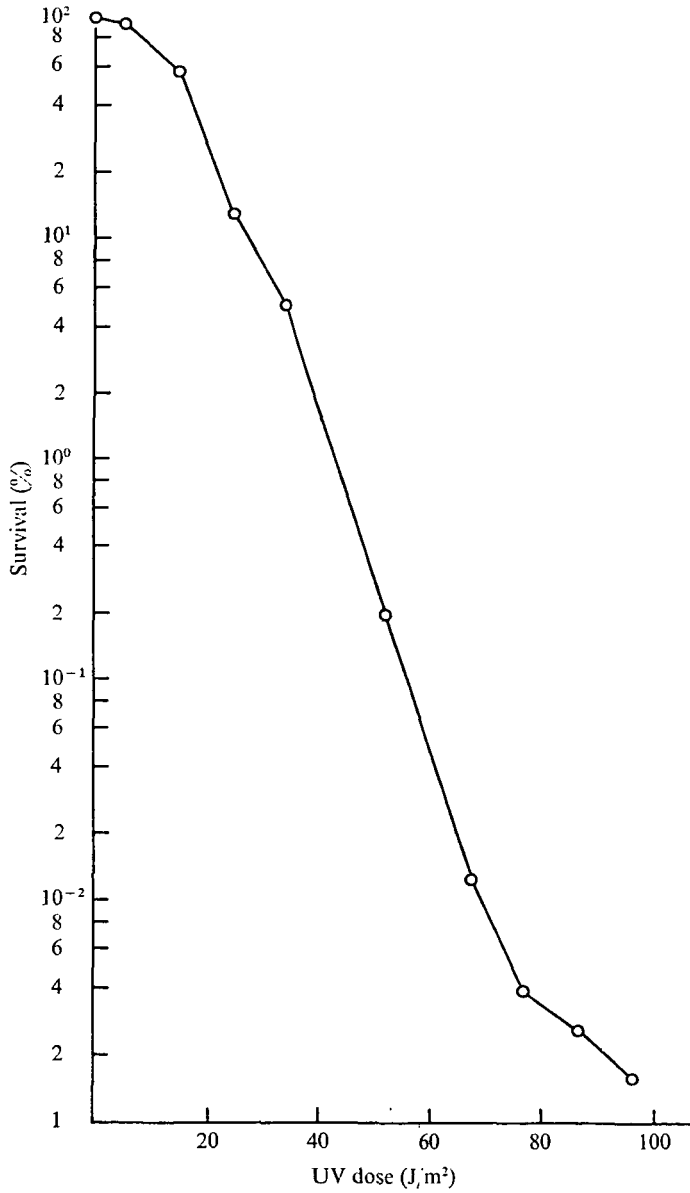

Fig. 1. *uvs*-2 survival curve.

The mutant isolates referred to in Table 1 failed to grow, or showed only limited growth when subcultured into minimal medium. These mutants were discarded after initial screening. The total number of conidia plated per trial varied between $1{\cdot}4 \times 10^4$–$11{\cdot}2 \times 10^5$. UV doses from 67·2 J/m² and 96·0 J/m² were exclusively employed since maximum reversions were previously found at UV survival values in this range (Nemerofsky, 1975).

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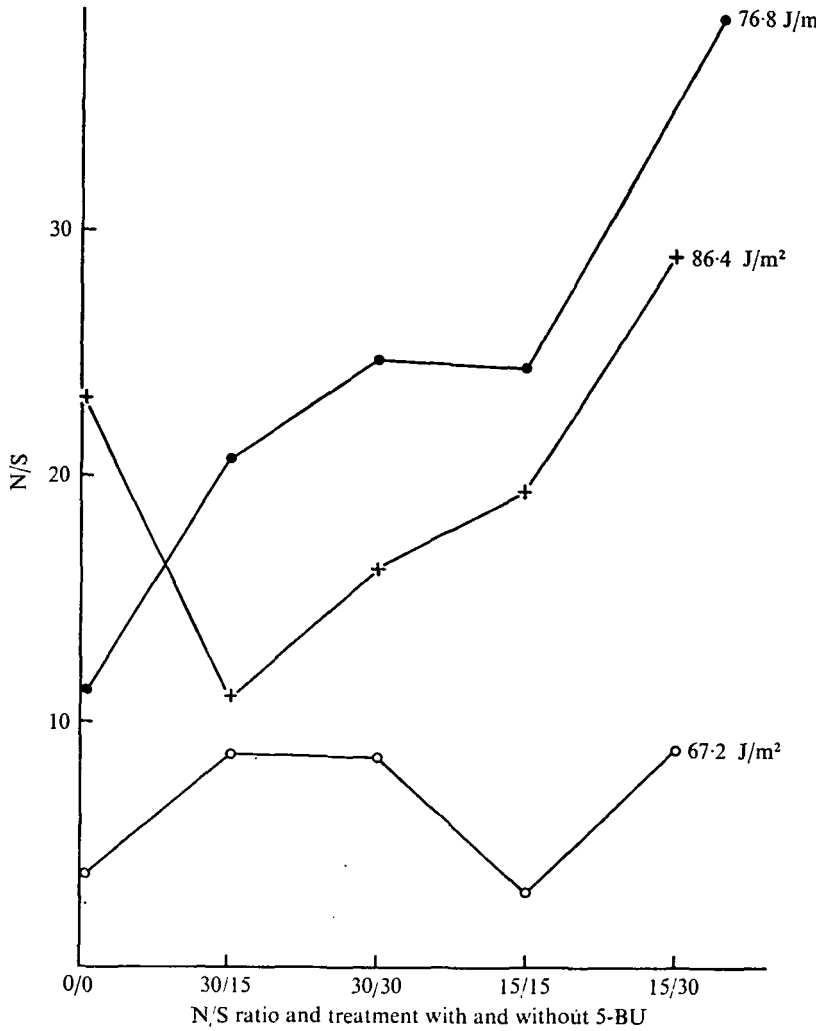

Fig. 2. N/S ratio and treatment with and without 5-BU. 0/0, no 5-BU: 30/15, 30 min pre-incubation, 15 min incubation with 5-BU; 30/30, 30 min pre-incubation, 30 min incubation with 5-BU; 15/15, 15 min pre-incubation, 15 min incubation with 5-BU; 15/30, 15 min pre-incubation, 30 min incubation with 5-BU.

The N/S value for conidia treated only with BU was determined according to the procedure described above. Conidia were collected, treated with BU for 15 min, and 45 min, washed and then plated on sorbose. The average of 3 trials for each exposure was $5{\cdot}7 \times 10^{-3}$ at 15 min, $4{\cdot}1 \times 10^{-3}$ at 30 min, and $5{\cdot}3 \times 10^{-3}$ at 45 min.

4. DISCUSSION

A similar interaction between UV and 5-BU treatments was demonstrated at the *rib*-1 locus in *N. crassa* which possesses an active dark repair mechanism (Nemerofsky, 1975). Since *uvs*-2 lacks a functioning dark repair system (Worthy &

Epler, 1973) and also shows an enhancement it seems possible that an error prone post-replication repair system exists in *N. crassa*. Worthy & Epler (1973*b*) indicate that certain aspects of DNA repair in bacteria and *N. crassa* are analogous. They also recognize that while their conclusions are tentative, and one has to be cautious when extrapolating from eukaryotes to prokaryotes, the evidence suggests the presence of a post-replication repair system in *Neurospora*.

The enhancement observed between the two mutagens may be due to the inefficiency of DNA repair in the presence of excess 5-BU. It would appear that the enhancement of mutation frequency in the current study indicates that the error prone mechanism is post-replication repair, since errors can only be introduced here. The greatest enhancement (Fig. 2) is observed when 5-BU is permitted to act the longest with the least time available for prior repair in the absence of 5-BU.

The current study relates the duration and extent of DNA repair in the absence and presence of 5-BU. The operation of the several repair systems can be considered as an interrelated series of events, such that each succeeding process repairs the remaining lesions. The extent to which mutations are induced reflects the accuracy of the individual repair process operating in a particular environment. It appears that the operational DNA repair system performs most accurately when the pre-incubation interval is maximum. Under the experimental condition established it would be expected that fewer mutations would be induced when the pre-incubation interval is maximum and BU exposure minimum since the pool of UV induced lesions would decrease. Alternatively a reduced pre-incubation interval and increased BU exposure should produce the highest frequency of mutations since DNA repair proceeds in a relatively large pool of unrepaired UV lesions. The sequence that demonstrates this is: 0/0, 30/15, 30/30, 15/15 and 15/30.

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REFERENCES

AOKI, S., BOYCE, R. P. & HOWARD-FLANDERS, P. (1966). Sensitization of *E. coli*, to radiation by bromouracil. *Nature* **209**, 686–688.

BOYCE, R. P. & HOWARD-FLANDERS, P. (1964). Release of UV light induced thymine dimers in *E. coli*. *Proceedings of the National Academy of Sciences, U.S.A.* **51**, 293–300.

BROCKMAN, H. E. & DE SERRES, F. J. (1963). 'Sorbose Toxicity' in *Neurospora*. *American Journal of Botany* **50**, 709–714.

CATCHESIDE, D. G., (1974). Fungal genetics. *Annual Review of Genetics* **8**, 279–300.

DE SERRES, F. J. (1971). Mutability of UV-sensitive strains of *Neurospora crassa*. *Genetics* **68**, s14–s15.

HOWARD-FLANDERS, P. & BOYCE, R. P. (1966). DNA repair and genetic recombination: studies on mutants of *E. coli* defective in these processes. *Radiation Research* (supplement 6), 156–184.

NEMEROFSKY, A. (1975). The interactive effect of UV irradiation and 5-BU at the *rib*-1 locus in *N. crassa*. *Canadian Journal of Genetics and Cytology* **17**, 275–278.

SCHROEDER, A. L. (1974). Properties of a UV-sensitive *Neurospora* strain defective in pyrimidine dimer excision. *Mutation Research* **24**, 9–16.

SETLOW, R. & CARRIER, W. (1964). The disappearance of thymine dimers from DNA, an error correcting mechanism. *Proceedings of the National Academy of Sciences, U.S.A.* **51**, 226–231.

STADLER, D. & SMITH, D. D. (1968). A new mutation in *Neurospora* for sensitivity to U.V. light. *Canadian Journal of Genetics and Cytology* **10**, 916–919.

TATUM, E. L., BARRATT, R. W., FRIES, N. & BONNER, D. (1950). Biochemical strains of *Neurospora* produced by physical and chemical treatment. *American Journal of Botany* **37**, 38–46.

WESTERGAARD M. & MITCHELL, H. K. (1947). *Neurospora*. V. A synthetic medium favouring sexual reproduction. *American Journal of Botany* **34**, 573–577.

WITKIN, E. M. (1966). Radiation induced mutation and their repair. *Science* **154**, 1345–1347.

WITKIN, E. M. (1969*a*). Ultraviolet induced mutation and DNA repair. *Annual Review of Genetics* **3**, 525–552.

WITKIN, E. M. (1969*b*). The role of DNA repair in recombination and mutagenesis. *Proceeding XIIth International Congress of Genetics* **3**, 225–245.

WORTHY, T. E. & EPLER, J. L. (1972). Repair of ultraviolet light-induced damage to the DNA of *N. crassa*. *Journal of Bacteriology* **110**, 1010–1016.

WORTHY, T. E. & EPLER, J. L. (1973*a*). Characterization of excision repair in *N. crassa*. *Journal of Bacteriology* **115**, 498–505.

WORTHY, T. E. & EPLER, J. L. (1973*b*). Biochemical basis of radiation-sensitivity in mutants of *Neurospora crassa*. *Mutation Research* **19**, 167–173.